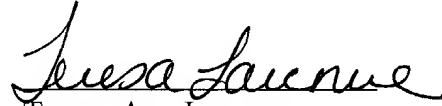


Amendment and Reply Under 37 C.F.R. § 1.116
Application Serial No. 09/242,561

Entry of these claim amendments as well as prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,
KENYON & KENYON

Dated: 3/20/03


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Please replace Table 2 starting on page 26 with the following:

Table 2

<u>reagent</u>	<u>final concentration</u>
KCl	50mM
Tris-HCl, pH 8.3	10mM
MgCl ₂	3.5mM
dATP	200 μ M
dCTP	200 μ M
dGTP	200 μ M
dUTP	400 μ M
β -actin forward primer (SEQ ID NO:1)	300nM
β -actin reverse primer (SEQ ID NO:2)	300nM
β -actin probe (SEQ ID NO:3)	200nM
AMPLITAQ DNA polymerase	0.25U/ μ l
AMPERASE UNG	0.01U/ μ l
human male DNA	0.2ng/ μ l

(Two types of samples, one containing human male DNA and the other containing no human male DNA, were prepared.)

Please replace paragraph 2 on page 39 with the following:

Fig. 32 is an exploded view of the holder containing the reaction vessels incorporated therein. The reaction vessels 100 are fixedly fitted in concavities of the holder body 130. After the fitting of the reaction vessels 100 in the holder 130, a fixing plate 133 is detachably attached on to an upper surface of the body with an easily releasable adhesive, VELCRO or the like. The fixing plate 133 is made of a thin metal plate having excellent thermal conductivity and has a role to fix the reaction vessels to the holder body 130. To increase heat transfer between the holder body 130, the reaction vessels 100, and the fixing plate 130, interstices therebetween are filled with oil or a grease. When placed in a thermal cycler, the holder is placed with the side of the fixing plate 133 in contact with a heat block.

Please replace paragraph 2 on page 40 with the following:

To prevent adsorption of an enzyme on an inner wall of an ink jet head, study was made on coating. As a system for optically detecting whether PCR proceeded or not, TAQMAN SYSTEM (produced by Perkin Elmer Co.) was employed. A reaction solution for PCR was prepared as shown in the following "Table 3."

Please replace Table 3 starting on page 40 with the following:

Table 3
Composition of Taqman PCR system
containing a human genome as a template

<u>reagent</u>	<u>final concentration</u>
KCl	50mM
Tris-HCl, pH 8.3	10mM
MgCl ₂	3.5mM
dATP	0.2mM
dCTP	0.2mM
dGTP	0.2mM
dUTP	0.4mM
β -actin forward primer (SEQ ID NO:1)	0.3 μ M
β -actin reverse primer (SEQ ID NO:2)	0.3 μ M
β -actin probe (SEQ ID NO:3)	0.2 μ M
AMPLITAG DNA polymerase	0.1U/ μ L
AMPERASE UNG	0.01U/ μ L
human male genomic DNA (produced by Boehringer Mannheim Co.)	1ng/ μ L

Please replace Table 5 on page 47 with the following:

Table 5

<u>reagent</u>	<u>final concentration</u>
KCl	50mM
Tris-HCl, pH 8.3	10mM
MgCl ₂	3.5mM
dATP	0.2mM
dCTP	0.2mM
dGTP	0.2mM
dUTD	0.4mM
β -actin forward primer (SEQ ID NO:1)	0.3 μ M
β -actin reverse primer (SEQ ID NO:2)	0.3 μ M
β -actin probe (SEQ ID NO:3)	0.2 μ M
AMPLITAQ DNA polymerase (4-fold concentration relative to one for a reaction in a tube on a usual scale)	0.1U/ μ L
AMPERASE UNG	0.01U/ μ L
target DNA (SEQ ID NO:4)	1ng/ μ L

Please replace Table 7 starting on page 50 with the following:

Table 7

<u>reagent</u>	<u>final concentration</u>
KCl	50mM
Tris-HCl, pH 8.3	10mM
MgCl ₂	3.5mM
dATP	0.2mM
dCTP	0.2mM
dGTP	0.2mM
dUTD	0.4mM
β -actin forward primer (SEQ ID NO:1)	0.3 μ M
β -actin reverse primer (SEQ ID NO:2)	0.3 μ M

β -actin probe (SEQ ID NO:3)	0.2 μ M
(20-fold concentration relative to one for a reaction in a tube on a usual scale)	
AMPERASE UNG	0.01U/ μ L
target DNA (SEQ ID NO:4)	1ng/ μ L

Please replace the first paragraph on page 51 with the following:

A sample were subjected to reaction under a thermal cycle of at 50°C for 2 min, at 95°C for 10 min, and under 40 times repetition of a cycle of at 92°C for 1 min, at 54°C for 1 min and 72°C for 2 min, and further at 72°C for 10 min, and observation with a fluorescence microscope was conducted. As a result of the observation, in comparison between the sample (+) subjected to the thermal cycle and a sample (-) unsubjected to the thermal cycle, change in fluorescence was clearly observed. The (+) sample showed a light green fluorescence and the (-) sample showed a reddish yellow fluorescence. The difference in fluorescence corresponded to the difference in fluorescence spectrum between a case where PCR proceeded and a case where PCR did not proceed when TAQMAN PCR was conducted in a usual tube. It is considered that PCR progressed in aqueous droplets in the (+) sample.

Please replace the second paragraph starting on page 51 with the following:

The reaction vessels of (+) and (-) samples were superimposed, and aqueous droplets in both the reaction vessels were microphotographed (taken) in one photomicrograph. As a film, a color slide film (EKTACHROME DYNA EX, IS0100, produced by Kodak Co.) was used. The image in the developed film was captured into a personal computer with a film scanner (Quick scan 35, manufactured by Minolta Co.,Ltd.) and analyzed by means of an image analysis software (PHOTO SHOP, produced by Adobe Systems Inc.). Arbitrary 30 points were taken (30 points were taken at random) in the fluorescence image of the aqueous droplets of the two samples, and color separation was conducted to obtain values of red (R), green (G) and blue (B). Ratios of R and G to R+G+B were plotted in Fig. 39. It is seen that the (+) sample and the (-) sample emitted evidently different fluorescences.

Please replace paragraph 2 starting on page 52 with the following:

7) Experimental Example

From the above-described studies, it was found to be advantageous that PCR be performed under the following conditions. Thereupon, PCR was performed under the conditions.

(a) use of a thin reaction vessel having an inside thickness of $50\mu\text{m}$ or less (which has a simple structure and is inexpensive) to prevent dissolution of a reaction solution in an oil

(b) vessel surface coatings capable of realizing both of prevention of adsorption of enzyme and prevention of scattering of aqueous droplets

(c) acceleration of PCR by increasing an amount of an enzyme

Please replace the second paragraph starting on page 53 with the following:

After completion of the reaction, all portions of the solution were collected into one in order to remove the Primers and the like, and the solution was purified by ultrafiltration. The solution was divided into portions in 6 tubular ultrafiltration membranes (MICROCON 1000, produced by Amicon Co.) and filtered by a centrifugal separator at 500G for 24 min. $300\mu\text{l}$ of TE buffer (pH 8.0) was added to each of the portions in the tubes, and the portions of the solution were further filtered by the centrifugal separator at 500G for 15 min and washed. The washing was conducted two times. $10\mu\text{l}$ of TE buffer (pH 8.0) was further added to each tube to dissolve the purified sample which remained on the ultrafiltration membrane. Fig. 40 shows results of analysis by means of 0.8% agarose gel electrophoresis. Lanes in Fig. 40 show results of electrophoretic migrations of the following respective samples.

lane 1: molecular weight marker ϕ X174/HincII ($2.6\mu\text{g}$)

lane 2: pre-ultrafiltration reaction solution ($10\mu\text{l}$)

lane 3: post-washing solution (first washing) ($10\mu\text{l}$)

lane 4: post-washing solution (second washing) ($10\mu\text{l}$)

lane 5: purified sample ($10\mu\text{l}$)